ANTIBIOTICS:
THE GREATEST TRIUMPH OF
SCIENTIFIC MEDICINE

The use of antibiotics has given us medical control of bacterial infections. This is a health standard that we have become accustomed to and have come to regard as self-evident. Today, it is impossible to imagine health care that is not able to cope efficiently with bacterial infections. Medical disciplines such as oncology and organ transplantation surgery would simply collapse without access to modern antibiotics.

The tremendous success of antibiotics in the field of infectious diseases for seven decades or so has led to very wide distribution and consumption of these agents. Besides their medical use for human beings and animals, antibiotics have been used in very large quantities as growth stimulants in husbandry and as prophylactic protection against plant pathogens. All this has led to the spread of millions of tons of antibiotics in the biosphere during the antibiotics epoch. This has induced a drastic environmental change, a toxic shock to the bacterial world. It has been said that “the world is immersed in a dilute solution of antibiotics.”
Bacteria have adjusted to the changed environment in the usual method used by living organisms: by evolution. The bacterial world, including human pathogens, has developed and mobilized molecular defense mechanisms for protection against the human-produced poisons that antibiotics are. This has led to increased antibiotics resistance among human pathogens, which are becoming more difficult to treat. This poses a serious threat to our health standard in that the ability of medicine to cope with bacterial infections has slowly been eroded. Medical journals and daily newspapers report on cases of infectious disease that were untreatable because of antibiotics resistance. One recent report described a young woman dying of tuberculosis despite intensive treatment. The tuberculosis bacteria causing the disease were multiply resistant and thus resisted treatment with all available antituberculosis drugs. What is happening, and what is going to happen?

Harmful cells comprise the two greatest threats to our health. In the first case, our own cells lose their growth regulation by genetic changes, thereby causing cancer. In the second, foreign organisms infect and establish themselves in the tissues of the human body, inhibiting their functions and destroying them by the action of toxins. Bacteria form the dominant part of the latter group: tuberculosis, syphilis, cholera, typhus, typhoid fever, and bubonic plague, for example. The medical treatment of cancer and that of bacterial infections are related in that both include the use of cell growth–inhibiting or cell-killing agents. Cancer cells are treated with cytostatics, which are difficult to use and must be handled by oncology specialists. This is because cancer cells originate from normal cells and are metabolically very similar to normal cells, letting cytostatics also interfere with healthy cells, such as those of the bone marrow, where the continuous growth of cells is necessary for the support of life.
SELECTIVITY

Bacteria belong to another biological world and are structurally and metabolically very different from our cells. They can be inhibited in growth and also killed by agents that do not interfere with our cells. That is, antibacterial agents, antibiotics, used for clinical purposes in medicine must act selectively on bacteria. Their handling can therefore be focused on the characteristics of the infecting bacterium.

Penicillin was discovered more than 80 years ago. Penicillin and its many followers, all with a selectively inhibiting effect on bacteria, had a tremendous impact on the treatment of infectious diseases and on their panorama of occurrence in the first decades of their ubiquitous clinical use (1950–1980). The great clinical success of antibiotics changed the attitude of the medical profession toward bacterial infections. This is reflected in a statement from 1969 by the Surgeon General, William H. Stewart, to the U.S. Congress: “It is time to close the book on infectious diseases.”

The surgeon general is the highest medical officer in the U.S. Department of Health and Human Services.

Antibiotics are unique among pharmaceutical remedies in that they do not direct their action toward our own cells but selectively toward foreign cells, bacteria coming from the outside and infecting our tissues. Their selective action means that they must target physiological and biochemical differences between our cells and bacterial cells in order to effect bacteriostatic or bactericidal activity. The key property of clinically useful antibacterial agents, then, is selectivity. It can be noted that in the search for new antibiotics in molds and other microorganisms, with Penicillium as an example, many selective and useful antibiotics were found (e.g., streptomycin and rifampicin), but also others with a good antibacterial effect but without selectivity, making them unusable for the clinical treatment of bacterial infections. The
latter antibiotics show inhibiting or killing activity toward both bacteria and our cells, and have in some cases (e.g., adriamycin, bleomycin, and mitomycin) found use as cytostatic agents in the treatment of cancer, and then under the usual strict oncologist control of, among other things, bone marrow function.

**DEVELOPMENT OF RESISTANCE**

Since antibiotics are only active against foreign cells, bacteria, and should have no effect on our cells and tissues, they are not pharmacologically active, except for side effects that occur with several of them when given in large doses. This means that they can be prescribed less strictly than other pharmaceuticals. In many patients showing signs of infection they are given simply for safety, without a strict bacterial diagnosis. This has contributed heavily to the very large consumption of antibiotics that can be estimated from sales figures, which can be used as good proxies for actual consumption (Chapter 2).

Resistance to antibiotics among pathogenic bacteria has developed within a short time and in many ways faster than could have been expected. This can be explained partially by the short generation time of bacteria, allowing them to undergo a Darwinian evolution in a much shorter time than has been possible for animals and other organisms. Furthermore, bacteria have the ability to manipulate their own genetic makeup, leading to a faster adaptation to the toxic effects of antibiotics: that is, the development of resistance. It can be looked upon as the natural genetic engineering of bacteria, including the uptake and incorporation of resistance-mediating genes from related organisms by adaptation of evolutionary old genetic mechanisms to the new environmental situation of the large presence of antibiotics. No microbiologist can escape feeling surprise and wonder as these phenomena continuously unfold.
Resistance is the dark and daunting side of the antibiotics triumph, and we are forced to realize that the health standard that antibiotics have given us is not stable. The great asset that antibiotics represent is devalued by the evolution of resistance. In a longer perspective this development is quite threatening. Many medical specialities are dependent on efficient antibiotics. Will we be able to maintain control of bacterial infections, or will our descendants look back nostalgically and talk about the time that we had both oil and antibiotics?

SULFONAMIDE: THE FIRST ANTIBACTERIAL AGENT ACTING SELECTIVELY

Louis Pasteur, a great French microbiologist of the nineteenth century, formulated and proposed what was called the germ theory of disease, the concept that infectious disease was caused by microorganisms. Later, Robert Koch at the Imperial Health Office in Berlin provided proof, with Bacillus anthracis as an example, that there is a definite causal relation of a particular microorganism to a particular disease. From these ideas Koch formulated his postulates for characterizing a pathogenic microbe:

1. The organism is found regularly in the lesions of the disease.
2. It can be isolated in pure culture on artificial media.
3. Inoculation of this culture produces the disease in experimental animals.
4. The organism can be recovered from lesions in these animals.

Based on these basic ideas, Paul Ehrlich at the Royal Institute for Experimental Therapy in Frankfurt am Main advanced the idea of direct selective action of a drug on infecting microbes. His
expression for this was the "magic bullet," which would exhibit a greater affinity for pathogenic bacteria than for host cells. For this selective action he coined the word chemotheraphy. Ehrlich further observed that dyes stained different cell components selectively and proposed the idea that organic stains taken up, particularly by living cells, could have a therapeutic effect by interfering with bacterial infections.

In the 1930s, these ideas led Gerhard Domagk, who was working at the Institute of Experimental Pathology at the I.G. Farbenindustrie in Elberfeld, Germany, to the discovery of Prontosil rubrum (4-sulfonamide-2',4'-diaminoazobenzol, Domagk 1935) (Fig. 1.1); a chemically synthesized dye of red color, which showed an effect against bacterial infections in animals. It was, however, inactive in vitro. Jaques and Therese Tréfoüel of the Pasteur Institute in France could show that patients treated with Prontosil excreted a simpler product, sulfanilamide, which was active in vivo as well as in vitro against the growth of bacteria. This was a dramatic development since it finally established Ehrlich's principle of chemotherapeutic action. Sulfanilamide is a colorless substance and not a dye, partly contradicting the theory leading to its discovery.

Sulfanilamide was set free from the dye by hydrolysis in vivo in animal experiments. Sulfanilamide was thus the first antibacterial agent to act selectively. The first trials of Prontosil rubrum on animals were performed by Domagk in 1932. He could show that mice infected experimentally with Streptococcus pyogenes by injection into the peritoneum were protected from peritonitis with this agent. The results were published in Deutsche medizinische Wochenschrift in 1935, and sulfonamides were soon used widely for the clinical treatment of infections with streptococci, staphylococci, meningococci, and other severely pathogenic bacterial agents. Domagk's work is unjustly forgotten today but was much appreciated by his contemporaries, and at the end of
the 1930s he was nominated for the Nobel Prize in Physiology or Medicine. The Nazi regime of that time in Germany had, however, declared that it did not want to see any German as a Nobel laureate, probably because of the Nobel committee’s choice of earlier Nobel Peace Prize laureates. The German government of that time tried through its embassy in Stockholm, and also directly through the foreign office in Berlin, to interfere with the work of the Nobel committee at the Karolinska Institute in
Stockholm. The Nobel committee, with its chairman pathology professor Folke Henschen, stood up to the pressure, however, and asked the medical faculty at the Karolinska Institute to award the prize to Domagk. In his memoirs from 1957, Folke Henschen, who personally knew Gerhard Domagk, mentioned that in the night following that day in October 1939 when the prize was announced, Domagk was arrested by Nazi soldiers in his home in Wuppertal and taken to jail. Next morning, when the prison director made his daily round, he met with Domagk, who did not seem to fit the environment. “Who are you?” he asked. “I am Professor Gerhard Domagk of the University of Münster.” “Weshalb sind Sie hier denn?” Domagk’s reply: “Ich habe den Nobelpreis bekommen.” The Nazi authorities did not allow Domagk to travel to Stockholm to receive the prize in December 1939. He did not go to Stockholm to receive the medal and the diploma until 1947, but because Alfred Nobel’s will specifies that the offer of prize money expires on the day of the award ceremony, he received no prize money.

Sulfonamides chemically synthesized beginning with Domagk’s Prontosil rubrum were widely used as efficient and inexpensive antibacterial drugs for the treatment of both gram-positive and gram-negative pathogens, and they had a deep impact on the fate of Europe. In December 1943, British Prime Minister Winston Churchill had just completed a complex series of meetings, among them the fateful conference with Franklin D. Roosevelt and the Soviet leader Joseph V. Stalin in Teheran. He was on his way to meet with the U.S. General Dwight D. Eisenhower in Tunis to discuss the D-day landings when he contracted a severe case of pneumonia. His doctor, Lord Moran, decided to treat his important patient with a new drug, a sulfonamide. The treatment was successful, and there is little doubt that the novel sulfa drug defeated the pneumonia and probably saved the life of this important European leader.
Chemotherapeutics and Antibiotics

The chemically synthesized sulfonamide was the first antibacterial agent to act selectively. The introduction of sulfonamide into clinical practice can be regarded as the birth of chemotherapy as defined by Paul Ehrlich. Through the years, however, the term chemotherapy has come to mean treatment with cytostatic agents in the treatment of tumors. The original distinction between chemotherapeutics, chemically synthesized antibacterial agents such as sulfonamides, and antibiotics produced by living organisms has been difficult to retain, not least because medicinal chemists have been increasingly skillful in modifying antibiotic structures: for example, to escape resistance development (Chapter 4). The term chemotherapeutics is not used much at present. Instead, the word antibiotics has come to comprise all selectively acting antibacterial agents, even though the meaning of the word is not altogether correct when applied to antibacterial agents such as sulfonamides, trimethoprim, and linezolide.

PENICILLIN: THE FIRST ANTIBIOTIC

Penicillin was the first antibiotic in the strict sense of the word: that is, an antibacterial agent produced in a living organism. The original observation was made by Alexander Fleming at the bacteriological laboratory of Saint Mary’s Hospital in London. In his research, Fleming was interested in staphylococci, particularly in the color and form of staphylococcal colonies on an agar plate. He had a hypothesis, which could never be verified, that there was a connection between the appearance of staphylococcal colonies and their pathogenicity. Among his staphylococcal plates, on one occasion, Fleming observed a plate with a large patch of mold growing on it (Fig. 1.2). The staphylococcal colonies on the same plate seemed to maintain a distance from the mold, not growing in its vicinity.
FIGURE 1.2 The discovery of penicillin. A replica of the original plate of Alexander Fleming showing a patch of _Penicillium_ mold and _Staphylococcus_ colonies seeming to avoid the mold patch.

This phenomenon caught Fleming’s attention, and one of the many biographies about him (Gwyn Macfarlane, _Alexander Fleming, The Man and the Myth_, The Hogarth Press, London, 1984) describes how on a sunny September morning in 1928 on the lawn outside the laboratory, he showed the plate to two fellow bacteriologists. None of the three could explain the phenomenon on the plate or at all imagine that at that moment they had a tryst with destiny. The interpretation of this phenomenon would open the way for the greatest triumph of scientific medicine: the control of bacterial infections with selectively acting drugs. The full impact of the observation was finally appreciated, and the original agar plate, showing antagonism between two microorganisms via a soluble agent, is now in the British Museum in
London. The diffusible agent inhibiting bacterial growth on the plate in the vicinity of the mold was named penicillin by Fleming, and together with its many derivatives, it would eventually become dominant among antibiotics in the treatment of bacterial disease.

There is another whim of destiny in the penicillin story. By its mechanism of action (Chapter 4), penicillin cannot act on resting nondividing bacterial cells—only on growing bacteria. This circumstance, together with the property of mold to grow much more slowly than staphylococci, led to the conclusion that penicillin could not have been discovered in the manner described. If the agar plate was already polluted with mold cells when Fleming streaked it with the staphylococci he was interested in, they would have grown out to be insusceptible to penicillin long before the mold had grown out enough to produce penicillin. The mold could also not have grown out to form a colony before inoculation with bacteria, since no microbiologist would use a contaminated agar plate. This microbiological mystery seems to be explained by a fantastic sequence of coincident circumstances. Fleming seems to have inoculated the agar plate at the end of the month of July and then left for summer holiday in Scotland, forgetting that the plate was on the bench and thus not placed in the 37°C incubator. The weather records for London from 1928 show that the first week of August that year was unusually cold, followed by hot summer weather. Mold cells grow faster than bacteria at low temperatures, which means that a mold colony could have formed during the cold spell, while the staphylococci caught up in the following warm period, then to meet with the penicillin produced and diffused out from the mold, forming the famous zone. This could be looked at as an example of serendipity, a scientist finding something quite significant without having looked for it (Fig. 1.3).
THE GREATEST TRIUMPH OF SCIENTIFIC MEDICINE

FIGURE 1.3 Sir Alexander Fleming celebrated by students at the University of Edinburgh.

The First Therapeutic Trial

Fleming identified the penicillin-producing mold as Penicillium notatum and showed that extracts from cultures of it inhibited the growth of several pathogenic bacterial strains. He also studied toxicity and therapeutic possibilities in animal experiments. Fleming left this research after about half a year, however, with a report delivered on May 10, 1929 and published in the June issue of British Journal of Experimental Pathology (No 3, volume 40). In this paper the therapeutic possibilities of penicillin are only mentioned in connection with the treatment of infected wounds. It is an enigma in the history of medicine why Fleming left research on penicillin so quickly. The most important reason for that was probably his observation that injected penicillin disappeared from the circulating blood of a rabbit within
half an hour, whereas test tube experiments needed longer to show the growth-inhibiting effect on bacteria. Fleming’s basic observations on penicillin were developed further toward an antibacterial remedy only after a period of 12 years, in 1940.

Rediscovery of Penicillin by a Basic Scientific Approach

In 1940, Australian-born Howard Florey, a professor of pathology, German-born Ernst Chain, a biochemist, and British-born Norman Heatley, a biochemist, all three at Oxford, England, began scientific studies on penicillin. Fleming had shown that penicillin interfered with the bacterial cell wall, and the three men wanted to investigate agents that had the ability to dissolve the murein of the cell wall in parallel with the enzyme lysozyme, the mechanism of action of which Florey had just studied. Chain first thought of penicillin as an enzyme, but very soon during purification, it emerged as a small molecule. After further purification the therapeutic possibilities could be discerned. It is interesting to note that it was purely a scientific interest in bacterial cell wall degradation that led the three scientists to take up study of the phenomenon discovered by Fleming. The realization of therapeutic possibilities led to what has been called the most important pharmaceutical experiment ever carried out. It began on Saturday morning, May 25, 1940, in Oxford when Howard Florey injected eight laboratory mice intraperitoneally, each with $10^8$ cells of *S. pyogenes*. One hour later, four of the eight mice were injected subcutaneously with 10 mg of a brown powder dissolved in water. At half past three on Sunday morning, all four of the mice injected with the brown powder solution were healthy and agile, whereas the other four were dead. The brown powder was penicillin, but only 0.1% of it was actually penicillin; 99.9% constituted impurities.
The experiment indicated that penicillin could be developed into an important medicine, and Florey and Chain, in collaboration with Norman Heatley, tried to solve the biggest problem at the time: to grow *P. notatum* in sufficient volume to be able to purify penicillin from the growth medium in medically usable quantities. Heatley was the co-worker who devised a purification method and also the method needed to assay penicillin activity. The resources of Oxford were limited because of World War II, but with help from the American pharmaceutical industry, production was begun. The penicillin produced quickly performed as a dramatically efficient remedy against bacterial infections. It immediately provided great relief in the treatment of infected war wounds, and very soon it found its way into clinical medicine in general. Alexander Fleming, Howard Florey, and Ernst Chain were awarded the Nobel Prize in Physiology or Medicine in 1945. Today, penicillin is produced in copious amounts all over the world using industrial procedures that are so efficient that the final product is pure enough for direct use in pharmaceutical products. The large global production of penicillin today has led to it being regarded as a commodity on the scale of coffee and tea. Organic chemists have succeeded in the total synthesis of penicillin, but industrial production today takes place in large tanks where penicillin-producing mold cells are grown. The original *P. notatum* has been replaced by *Penicillium chrysogenum*, which is a more efficient producer.

**Betalactams**

The penicillin isolated originally, penicillin G, is acid labile and has to be administered parenterally lest it be destroyed by stomach acid. One of the first important derivatives of penicillin G was phenoxymethylpenicillin (penicillin V), which is acid stable and can be given by mouth. The characterization
of the betalactam ring of penicillin as the active component of the molecule has extended this group of antibiotics to a large family, the betalactams, including penicillins, cephalosporins, and monobactams (see also Chapter 4 and Fig. 1.4). The incitement for finding all these betalactams has been both to find antibacterials against pathogens with varying susceptibilities to betalactams, and to counteract the development of resistance (see also Chapter 4).
STREPTOMYCIN: THE SECOND ANTIBIOTIC IN THE HISTORY OF ANTIBACTERIAL AGENTS

The tremendous success of penicillin as an antibacterial agent isolated from a living organism induced an intensive search for further antibiotics among other microorganisms. Selman Waksman at Rutgers University was a well-known expert on soil microbes at the time (Fig. 1.5). Waksman was particularly interested in the antagonism between microorganisms as a means to understanding how soil microbes interact. It was said that one morning around 1940 he exclaimed to his collaborators: “Stop

FIGURE 1.5 Selman Waksman, discoverer of streptomycin. In this photograph note the burn hole in the elbow of the lab coat, which is often characteristic of microbiologists, caused by the small, easily overlooked ignition flame of a bunsen burner.
what you are doing. Look at what those English can do with a mold. I know that organisms in the soil can do a lot more—let’s start looking.” Waksman’s laboratory, among others, started the search for antibacterial agents among soil microorganisms. He and his co-workers concentrated their search on *Actinomyces* species and very soon found the two antibacterials actinomycin and streptothricin, which were, however, too toxic for use as antibacterial remedies. Actinomycin was toxic because it did not act selectively. Later it was put to good use as a cytostatic agent in the treatment of certain fast-growing forms of cancer, such as the epithelioma of the chorion, and streptothricin has been used for veterinary purposes in some parts of the world.

Further research by the group at Rutgers University resulted in the finding of streptomycin, which has been regarded as the second great antibiotic after penicillin. It had a dramatic medical impact because it was the first effective agent against *Mycobacterium tuberculosis* and thus the first effective remedy for tuberculosis, against which penicillin is not effective. The discovery was published in 1944 in *Proceedings of the Society for Experimental Biology and Medicine* in a paper, "Streptomycin: a Substance Exhibiting Antibiotic Activity Against Gram Positive and Gram Negative Bacteria.” The first author of the paper was Albert Schatz, a graduate student of Waksman’s. He had isolated one of the streptomycin-producing strains of *Streptomyces griseus* and also tested the effect of this new antibiotic on different bacteria. He would, however, not have been able to do this without access to the expertise on soil microorganisms and the system of methods available in Waksman’s laboratory. The discovery of streptomycin was not at the time regarded as anything genuinely new in the scientific world, but as a development of concepts formulated in the breakthrough of penicillin as a medicine. Streptomycin won fame, however, as the first remedy against tuberculosis, and Waksman was awarded the
Nobel Prize in Medicine or Physiology in 1952. It was awarded to Waksman alone. Although Albert Schatz was responsible for the actual discovery, he was not included in the prize. This and the substantial amounts of royalty money that the commercial distribution of streptomycin as a pharmaceutical would bring in led to one of the bitterest feuds the world of science has ever seen. It continued for more than two decades, and included lawsuits, most of which Waksman won.

The protocols and regulations of the Nobel Committee at the Karolinska Institute are kept confidential for 50 years, so those regarding Waksman are now accessible for scrutiny. The streptomycin discovery, particularly with reference to the treatment of tuberculosis, was the subject of several reports and evaluations at that time. The most important one was dated August 21, 1952, and was signed by Einar Hammarsten, then professor and head of the Department of Medical and Physiological Chemistry at the Karolinska Institute. Hammarsten expressed himself very clearly, specifying that the discovery of streptomycin belonged to Waksman alone. He argued that the first streptomycin-producing strain of *S. griseus* was isolated by Waksman and that he had worked out the procedure for the isolation of streptothricin, which was also used for the original purification of streptomycin. The most important early publications on streptomycin carry many young authors’ names, including Albert Schatz, Elisabeth Bugie, and Boyd Woodruff. Hammarsten wrote that these young co-workers could not be included as prizewinners.

**The First Remedy for Tuberculosis**

Streptomycin was the first efficient remedy against tuberculosis, and it quickly reduced mortality from this disease. It soon turned out, however, that it had severe clinical side effects. The most important were toxic effects on the sensory cells in the cochlea and
vestibulum, in many cases leading to deafness and to interference with body balance. The precise toxic effect of streptomycin is directed toward the sensory cells registering the pressure changes of sound, and seems to be mediated by its binding to the melanin in cochlea. Nowadays these clinical problems with side effects can be handled by using drug combinations and by carefully following the serum concentrations of the drug in combination with close observations of hearing ability.

CONCLUSION

Sulfonamide, penicillin, and streptomycin were the harbingers of the antibiotics era. The promises of these agents regarding effective control of all types of bacterial disease have largely been fulfilled by the broad array of antibacterial agents now available. Today, it is difficult to imagine the fear and anxiety connected to the earlier panorama of infectious disease. There is evidence from world literature: for example, a famous novel from 1924, *Magic Mountain* by Thomas Mann, later earning its author the Nobel Prize in Literature. In that great novel there is a description of the relentless progress of tuberculosis despite many, often very painful treatments given the patients at Berghof, a rather luxurious sanatorium in the Swiss Alps. The description is frightening and fearful to us today, who feel relief at an x-ray diagnosis of tuberculosis—because the aberrant structure seen on the screen is not cancer. Tuberculostatic agents promise healing within months, and in the Western world, the number of deaths in tuberculosis has decreased about 1000-fold since 1900.

Is it possible to come to grips with antibiotics resistance, or are we on our way back to an inability to handle pathogenic bacteria? In actuality there is no real reason for fear. Most bacterial infections can still be treated efficiently, but there are many serious difficulties on the horizon.